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REDUCING THE PERIOD OF SEXUAL REST FROM 5 TO 3 DAYS IMPROVES SEMEN QUALITY IN THE POINTER BREED OF DOGS

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ABSTRACT

The study investigated the changes in fresh semen quality parameters by reducing the period of sexual rest from the usual 5 days to reduced 3 days in the Pointer breed of dogs. Ten healthy male dogs were selected and were randomly divided into two groups i.e. group A and B with sexual rest of 3 and 5 days, respectively. Arousal/collection time, volume and pH were evaluated for all the three fractions of dog semen, whereas, only sperm rich fraction (SRF) was tested for motility, concentration, morphology and live spermatozoa percentage. Semen collection technique and environmental conditions were kept the same for all the dogs. All the three fractions were collected separately but for homogenous analysis, only SRF was focused for all the parameters in the experiment. Results revealed that sperm concentration was altered significantly in group A 410.880 $x10^6$ than group B 461.980 $x10^6$ ($P < 0.05$) while slight changes were observed in volume (group A 2.2 and group B 2.5 ml) and motility (81.860 and 80.340% in group A and Group B respectively). However, negligible effects were seen in pH, live and normal sperm cell percentages. The concentration was reduced in dogs ejaculated after 3 days rest but monthly total sperm count was increased around 18%. Live and Normal sperm percentage was also increased with reduced rest. Improvement in sexual behavior at the terminal stage of the experiment was an interesting observation. Collectively these findings indicate that overall quality of semen improves by reducing the rest time between the ejaculates.

Key words: Pointer dog semen, ejaculate frequency, sexual rest, seminal parameters, dog semen

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INTRODUCTION

The dog has been a part of human society since 4000 BC. Owing to the prevailing security environments, the use of dogs in rescue operations is a common practice. Increased demand of dog as a biological sniffing tool compels the preservation of good quality genes to maintain the desired traits for a longer period. Reproductive studies and experiments have made it possible to preserve a perfect gene by cryopreservation and fertilizing ova through artificial insemination (A.I.) within the female genitalia. In Europe, more than half of the A.I. approximately 50-55% in dogs is performed using fresh semen followed by frozen 35-40% and chilled semen 10%(Linde-Forsberg, 2001). Male fertility appears to be one of the major issues in dogs as approximately 20–25 % of breeding dogs do not fulfill the level of basic requirements of successful reproduction (Veznik *et al.,* 2004).

Semen in dogs is ejaculated in three fractions. Maximum spermatozoa are ejaculated in the intermediate fraction called sperm rich fraction (SRF) (Mascarenhas *et al.,* 2018). The first and third fractions are mostly of prostatic origin. Prolonged contact of spermatozoa with seminal plasma is associated with decreased motility and sperm viability (Rota *et al*., 2007). The second fraction is mostly collected separately for insemination with fresh semen, however, if the separate collection is not possible, the centrifugation is an alternative method being in practice. Separate collection of all the three fractions was adopted in this experiment. Fertility of an ejaculate is tested before cryopreservation either by crossing a number of bitches with a specific dog or evaluation of seminal characteristics of fresh semen by adopting different lab procedures. The former procedure is time consuming and results could be tainted due to the influence of bitch reproductive health. There appears to be a consensus among the scientific community on the adoption of lab procedures. Normal morphology and progressive motility of spermatozoa are considered the criteria for optimum fertility in male. Furthermore, the sperm motility is a preferred quality indicator in dog semen ejaculates (Rigau*et al*., 2001;Veznik*et al*., 2003). More than 70% motility is standardized for the viability of canine semen (Sicherle *et al*., 2020)

In dogs, there have been few studies of the ejaculatory frequency upon semen characteristics such studies are important as a dog in future is going to be presented for semen collection and cryopreservation. Foote (1964) reported that as a general rule, five days of sexual rest should be allowed before semen collection and evaluation (Gharajelar *et al*., 2016). On the other hand, if the dog is not ejaculated within 10 days, the adverse effects of sperm cell ageing are seen in the first

ejaculate obtained following prolonged sexual rest by increasing number of abnormalities, such as detached heads and distal cytoplasmic droplets in spermatozoa (Christiansen, 1984). Approximately 70% or more spermatozoa are obtained with two ejaculates per day as compared to a single collection.

Several studies are available on this aspect in various other species but a dog, having different reproductive physiology and breeding behaviour, still requires more research as the available work is comparatively not sufficient to the requirement. The study was therefore designed to evaluate the effects of reduced sexual rest in dog on fresh semen parameters with the ultimate goal of cryopreservation of desired genes by getting maximum benefits by using minimum inputs.

MATERIALS AND METHODS

Selection of Dogs: Ten healthy fertile dogs, ranging between 18 to 30 months of age were used in the experimental study. Dogs were kept under observation for any sickness till completion of the experiment and history of each dog was sought to know the sexual soundness (Araujo *et al*., 2020). Dogs were weighing between 25 – 30 kg and were maintained under same feeding, housing and environmental conditions. Dogs were divided into two groups (5 in each group A and B) to evaluate the effects of sexual rest.

Environments: Semen was collected in a dust-free, silent, and comfortable environment. Dogs were ejaculated in presence of a teaser bitch (Pignataro *et al*., 2020). Fifteen collections of each dog were assessed and compared. Semen from both the groups was collected in the morning between 0800 to 0900 hrs. The temperature of the collection room was maintained at 20ºC (Kutzler, 2005). Three handlers were used for the collection procedure and all other disturbing elements / factors were eliminated from the room to avoid interruption in the collection phase. Windows were covered with blinds to avoid external interference. Soothing light was available in the room.

Semen collection: Ejaculates were obtained by a manual massage using a siliconized latex cone attached with a plastic graduated tube (YUBI *et al*., 1987;Linde-Forsberg and Eneroth 2005;England and Heimendahl 2010;Farstad 2010;Lucio, Regazzi *et al*., 2016).Dogs were divided into two groups based on collection frequency at the sexual rest of 3and 5 days for group A & B respectively. The ejaculates were collected into a pre-warmed calibrated plastic tube(Rodenas*et al*., 2014) at 37^0 C without lubricant to evade spermicidal effects (England 1992;Freshman 2001).Semen collection area and analysis lab were adjacent rooms to reduce the effects of the time factor and thermal shocks.

Semen Evaluation: Equipment necessary for collection and evaluation was maintained at 37ºC (Christiansen 1984;Linde-Forsberg 1991; Feldman *et al.*, 1996; Feldman and Nelson 1996). The evaluations were carried out for arousal and collection time (seconds) (İnanç *et al*., 2018), semen volume (ml), semen pH, sperm motility (%), sperm concentration (n x 10^6 / ejaculate), live sperm (%) and morphologically normal sperm (%).Tests were carried out immediately after collection. Arousal time was measured from the exposure of dog to the massage till the first drop of semen, followed by the estimation of time of each fraction separately. Semen was observed macroscopically for any contamination of blood, urine or debris. Volume was measured in pre-warmed graduated plastic tubes to avoid thermal shock. Semen samples were then evaluated at a minimum criterion of 70% sperm motility (Hesser*et al.*, 2017) and morphologically abnormal sperm cells less than 30% (Hidalgo *et al.*, 2018). Semen for motility was evaluated at 37° C(Belala *et al.*, 2016)on a glass slide with cover slip under the objective power of 20xand 40x. Dilution was made by the same quantity of prostatic fluid (third fraction) to permit visibility of motile spermatozoa. Sperm concentration was evaluated by improvised Neubauer chamber (Dziekońska *et al*., 2018) and the number of sperms were counted in the pre-diluted SRF (sperm rich fraction) in part of the semen. The similar method reported by Ray *et al*. (2019). The percentage of the viable and morphological evaluation carried out by using Eosin – Negrosin stain under the 100x magnification i.e. oil immersion lens (Hori *et al.*, 2014). The number of spermatozoa was counted in 100 to get a percentage. Three slides were analyzed for each sample and average has been noted for the results. The data was analyzed using the following statistical model:-

$$Y_{ij} = \mu + P_i + E_{ij}$$

where Y_{ij} is a measurement of semen parameter of the jth animal for ith period of sexual rest; μ is the overall population mean; Pi is the fixed effect of an ith period of sexual rest (two levels i.e. 5days and 3 days), and E_{ij} is the random residual associated with each record. Residual effect for each trait was assumed to be distributed as N(0, Iσe2) and N(0, Iσhys2), respectively. Probability level was kept at 0.05 level for this experiment.

RESULTS AND DISCUSSION

Sexual behavior is a voluntary process and cannot be forced, so the dogs were allowed to approach the bitch freely. Dogs with little variations in sexual behavior and arousal time showed sniffing / licking of the hind-quarter and external genitalia of bitch. Some of the dogs did not mount and started giving semen. Salivation and mastication of teeth were signs of stimulation in male

dogs. Penile erection was ensured to get good semen quality.

The resulting effects of a reduced period of sexual rest on various semen parameters in pointer dogs are given in Table 1. For these pointer dogs, arousal time was calculated 93s (±1.41 SD) for group A and 86.04 (±1.48 SD) for group B with P-value < 0.0001. All three fractions were separated with distinct signs. It was observed that dog would thrust vigorously several times and during this phase first and second fractions were collected. It was further noted that pulsation was an indication of the third fraction. The collector would replace the tube for collection of the third fraction in a separate tube when pulsation would start. Quick reflexes of the collector were required to replace the collection tube to collect all the three fractions separately without disturbing the dog. In this experiment, the average time of collection was 39, 77, and 454 seconds for the first, second and third fractions respectively in both groups (A&B). It was also observed that if collection cone would touch the penis frequently, erection would subside and the dog would stop giving semen. Libido remained similar at both the occasions but overall improved at the terminal stages of the experiment.

Statistically, a significant difference was found for semen volume between both the groups. Semen volume was higher in group B. Ejaculate volume varies from breed to breed, depending upon the size of the testicle, age, health and physical condition of the dog. Overall volume depends on the amount of the third fraction, which typically constitutes 95% of the total ejaculate (Farstad, 2010). The average volume in SRF of group A recorded2.2 ml and in group B2.5 ml with a P-value of 0.0071. The volume was slightly higher in group B and lower in group A. The total volume of un-fractioned semen in the present study was found 18.8 ml in group A and 21.7 ml in group B. High values of volume in group B, indicate that sexual rest influences the results in a canine when the duration is reduced. Similar observations have been indicated by Hewitt and England (1999).

Table 1. Effect of reduced period of sexual rest on various semen parameters in pointer dogs.

Parameter	Group A (3 Days rest)			Group B (5 Days rest)			P-values
	Mean	SD	SEM	Mean	SD	SEM	
Arousal Time (seconds)	93.6	1.412	0.632	86.040	1.478	0.662	<0.0001*[1]
Collection Time (seconds)	77.520	3.245	1.451	73.680	1.660	0.743	0.0463*
Volume (ml)	2.200	0.122	0.055	2.500	0.141	0.063	0.0071*
pH	6.140	0.055	0.024	6.160	0.055	0.024	0.5796
Motility (%)	81.860	1.092	0.488	80.340	0.378	0.169	0.0187*
Concentration ($x10^6$)	410.880	19.099	8.541	461.980	1.008	0.451	0.0003 *
Live %	78.940	0.792	0.354	77.800	0.412	0.184	0.0214*
Normal (%)	79.5000	0.308	0.138	78.100	0.308	0.138	0.0001*

[1] Probability values with * symbol show difference (P < 0.05)

Check and Chase (1985)indicated that short interval ejaculation may improve semen quality in the oligospermic men. These workers also found that a similar effect may occur in some normal men following a short interval repeated ejaculation, although in general, spermatozoa concentration and semen volume decline but volume is not correlated with the semen quality in the dog. According to Johnston (1991), normal dog semen ranges in volume from 1 to 30 ml. Some dogs tend to urinate before ejaculation and add few drops to volume causing urosemina (urine contamination) which is undesirable. Schäfer *et al*. (1997) observed the changes in the volume of canine semen, collected twice a week for six months.

Kustritz (2007)observed the normal percentage of motile sperm in the ejaculate of normal dog should be more than 70 %. This was in agreement within the case of both the groups in the present experiment with an average around 80-81% motile sperms. The dog having sexual rest of 3 days had motility 81.86% (±1.09 SD) as compared to group B with 80.34%(±0.38 SD).Reducing the sexual rest shown improvement in motility with P-value equal to 0.0187 (Fig 1). A normal healthy dog has good motility percentage, however, a decrease in sperm motile percentage may result from temperature shock, contamination from water, blood or urine and also from long sexual abstinence or some infectious diseases.

The concentration of sperms was significantly higher (P=0.0003) in group B than group A. Calculated range was around 300 – 400 million which is closer to the range studied byJohnston (1991), who reported range between 300 – 2000 million sperm per ejaculate. The exact range of total spermatozoa in dogs ejaculate is not specific but any number less than 100 million sperm in semen sample usually means the dog's health issues are affecting the fertility (Eldredge *et al.,* 2007). The concentration depends on the testicular size and decreases with frequent semen collection, presumably as

epididymal reserves are depleted (England 1999). Normal dogs may ejaculate oligospermic or azoospermic samples due to anxiety or pain (Rodríguez-Martínez, 2006).

Amann (1986) also reported the fact that the number of sperm per ejaculate may vary according to age, testicular weight and sexual activity. A marked difference was observed in the concentration of both the groups (Table 1). Semen collected in group "B" (5 days sexual rest) was denser. The sperm per ejaculate count was lower in group "A". Nonetheless, for the collection of one calendar month, the sperm concentration increased by around 18% as more ejaculates (N=8) were collected by reducing the rest in one month from Group "A" as compared with group "B".

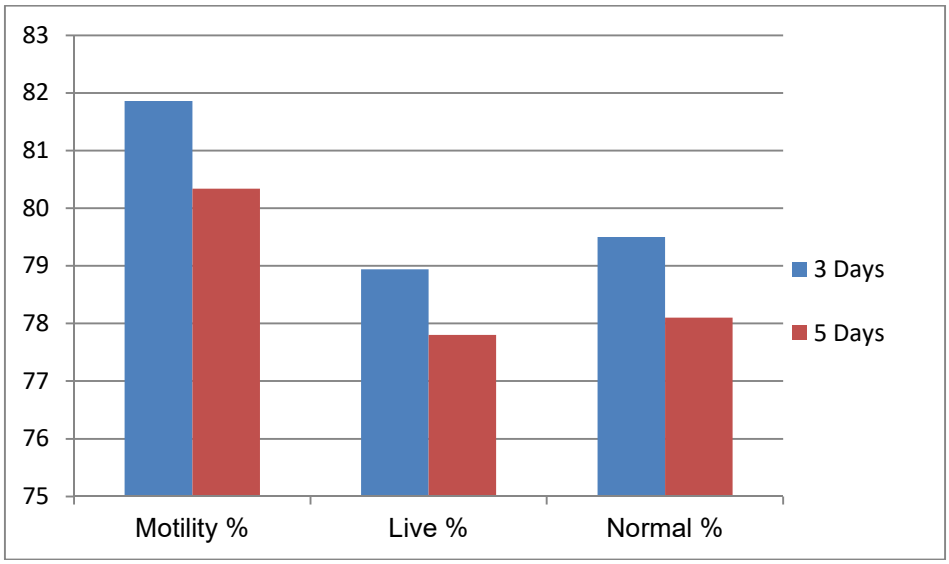

Fig 1

Similarly, live percentage of sperm cells was also improved with reduced sexual rest. The values were statistically significant with P-value 0.0214. Flushing and regular collection promote the regeneration of sperm production from seminiferous tubules. The values of live sperm were improved in Group "A" (79.50%) as compared to Group "B" (78.10%) with P-value equal to 0.0001. Similar observations were recorded for the normal percentage of sperm cells.

Conclusions: The present study shows that various important seminal parameters like motility, concentration, live and normal percentage are improved by reducing the sexual rest from five to three days in dogs. At the same time, a decrease is observed in volume and collection/arousal time without affecting the libido. Specifically, it could be concluded that reducing the gap between ejaculation improves the semen quality at the cost of increased times of arousal and collection. However, the minimum time of sexual rest required for a dog to retain good semen is maintained at 3 days in the present study. The study could be reproduced with a large data set to increase the validity of the results obtained in the present study.

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